TECHNICAL NOTE

SOME OBSERVATIONS ON THE ULTRAVIOLET IRRADIATION OF MILK (CENTRIFILMER PROCESS) WITH EMPHASIS UPON ORGANOLEPTIC EFFECTS AND SPORICIDAL EFFICIENCY [1]

The belief has long prevailed that milk does not lend itself to preservation by ultraviolet irradiation, both because of the low penetrating power in this medium and because of undesirable off-flavors induced in milk by ultraviolet radiation. Somewhat at variance with this view are the rather recently published findings of Albrecht *et al.* (1), who reported that mercury resonance (ultraviolet) radiation caused virtual sterilization of raw whole milk without producing undesirable off-flavors. With the development of the Centrifilmer, it became possible for the first time to produce a smooth uniform microfilm of moving liquid which, when exposed to suitable ultraviolet radiation, greatly increases the efficiency of sterilization. This fact, plus the established usefulness of the Centrifilmer in the processing of blood plasma and in the production of polio vaccine, led us to make an exploratory study of the Centrifilmer Process as applied to the preservation of milk. The centrifugal-filmer (Centrifilmer) consists of a vertical 15-in. stainless steel bowl, the inner wall of which flares outward at a 1° angle. Suspended inside the bowl are six water-cooled ultraviolet lamps (General Electric GL816: over 90% of the total lamp output is at 2537 Å). The lamps can be operated at two intensity levels, with total irradiance at the film surface approximately 7.22×10^4 ergs cm.$^{-2}$ sec.$^{-1}$ for Level I, and 10.83×10^4 ergs cm.$^{-2}$ sec.$^{-1}$ for Level II (1.5 times Level I). Sometimes a level of about one-third of Level I was used with two lamps. In operation, fluid from the inlet container is propelled by a metering-pump through a bubble trap and flow meter into the base of the bowl which rotates at a fixed speed (1,750 r.p.m.). By centrifugal action, the liquid moves up the walls of the bowl in a micro-thin film past the radiation source and is discharged at the top in separator fashion. In our experiments, the flow-rate was 100 ml/min, film thickness approximately 15 μ, exposure time 1 sec. Detailed description of this apparatus and its operation has been given by Benesi (3) and Taylor *et al.* (4).

Organoleptic results. The machine was arranged to collect sterile samples via a closed system of rubber tubings and glass Y's. Preliminary runs showed that this system, without separating the ultraviolet lamps, imparted a rather strong off flavor to milk. This was largely eliminated by replacing the rubber with tygon tubing and collecting the samples in small Erlenmeyer flasks directly from the outlet spout of the machine. This simplified system was used for flavor experiments. Treated samples and controls were evaluated by a six-person taste panel for preference sequence. Irradiation produced intense off-flavors in milk, varying from cooked-and-burnt-cabbage, scorched, burnt-feathers, irradiated, cardboard, etc., which increased in intensity with increase in irradiation dose. However, even at the lowest level of radiation (I with two lamps) the milk was unacceptable to critical judges. Irradiated flavor was more pronounced in pasteurized skim than in pasteurized homogenized whole milk.

A series of experiments was carried out under various conditions with commercially pasteurized homogenized milk, at radiation Level I. Irradiation in air decreased the off-flavor as compared to irradiation in nitrogen. Irradiation in CO_2 at normal or pH 5.0 did not eliminate off-flavor. Deaeration for 1 hr. in a vacuum at 40° C., with a small amount of nitrogen continuously bubbling through the milk or preheating at 75° C. for 0.5 hr., alone or in combination, increased off-flavor formed by subsequent irradiation in nitrogen. Preheating also increased radiation-induced off-flavor in air. Addition of 0.01% propyl gallate, NDGA, or glyoxal (a sulphydryl protecting agent) diminished off-flavor induced by radiation in nitrogen to such an extent that these samples were superior to a control irradiated in air, which in turn was better than the control irradiated in nitrogen. A sample with 0.1% added H_2O_2 irradiated in air rated poorer than the control irradiated in nitrogen. Best results were obtained with propylgallate irradiated in nitrogen, but the flavor was deemed unacceptable by the judges; in contrast, this compound had little or no effect if the milk was irradiated in air.

Several attempts were made to remove radiation-induced off-flavor once formed. Deodorization of irradiated milk for 1 hr. in a vacuum at 40° C., with a small amount of nitrogen continuously bubbling through the milk or heating at 70° C. for 15 min., did not improve the flavor. Addition of 0.1% NaHSO$_3$ increased off-flavor. Addition of 0.01—0.1% H_2O_2 after irradiation decreased the initial off-flavor considerably after storage in a refrigerator for from one to two days but not enough to win acceptance by the judges.

Further study of the flavor reactions did not seem justified in the light of the bacteriological

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[1] This study was conducted at the Western Utilization Research and Development Laboratory, Albany, California.

[2] Spores of *C. botulinum* were detoxified by heating at 75° C. for 10 min.

TABLE 1

Survival of bacterial (spores) in commercially pasteurized whole milk treated with ultraviolet radiation in the Centrifilmer[a]

Date	Inoculation	Intensity level*	Before Irradiation		After irradiation (in air)		
			Total (bacteria)	Spores	Bacteria (spores) (5.5 ml. tested)	Incubated 8-ml. samples good/bad	Incubated 300-ml. sample
			(per ml. × 10³)				
1958							
5/17		0	4.0	0.017	0	8/2	[e]
5/8		0	4.0[d]	0.013	0	9/1	....
5/13	Bacillus subtilis (spores)	I	....	1,300.0	0.0001	5/1	Spoiled
5/13	Bacillus subtilis (spores)	II	....	1,300.0	0.0001	6/0	Spoiled
5/13	Bacillus subtilis (spores)	I	....	16.0	0.015	5/1	....
5/13	Bacillus subtilis (spores)	II	....	16.0	0.0002	6/0	Spoiled
5/14	Clostridium botulinum (spores)	I	....	28.0	0	5/3[b]	Spoiled
5/14	Clostridium botulinum (spores)	II	....	38.0	0	9/0[c]	Not observed
5/16	Bacillus megatherium (spores)	I	....	370.0	0.86	0/5	Spoiled
5/16	Bacillus megatherium (spores)	II	....	370.0	0.58	0/5	Not observed
5/14	Bacillus stearothermophilus (spores)	I	....	2,700.0	2.1	Not observed	Not observed
5/14	Bacillus stearothermophilus (spores)	II	....	2,700.0	1.1	Not observed	Not observed

* I. 7.22×10^4 ergs/cm.$^{-2}$/sec.$^{-1}$—total incident irradiance at film surface. II. 10.83×10^4 ergs/cm.$^{-2}$/sec.$^{-1}$—total incident irradiance at film surface. [a] Flow rate 100 ml/min thickness of milk film approximately 15 μ, time of exposure 1 sec. [b] Spoilage caused not by C. botulinum but by aerobic spore-former. [c] 6-wk. observation. [d] No count. [e] No change.

results. Since radiation is relatively ineffective against many milk enzymes, radiation processing of milk would necessarily entail a supplemental heat treatment sufficient to inactivate lipase and other enzymes capable of causing deteriorative changes in the product. It was observed that heat sufficient to inactivate lipase in milk intensified the off-flavor which developed during subsequent radiation.

Microbiological results. Spores of four different bacterial species were used as test organisms: Bacillus subtilis (15U), representative of a type which has given much trouble in milk-distribution plants and condensaries; Bacillus megatherium (753), less resistant to heat than B. subtilis, but more resistant to ultraviolet; Bacillus stearothermophilus (1518), flat-sour type; Clostridium botulinum (62A), toxin-forming anaerobe considered to be the most radiation-resistant (gamma radiation) of all spore-forming species.

Well-washed stock spores,[2] suitably diluted and distributed in distilled water, were added to milk in Erlenmeyer flasks (capacity about two times that of volume of milk). The flasks and milk were shaken sufficiently to effect an even distribution of the spores in the milk, as judged by preliminary tests. The temperature of the milk when inoculated, and subsequently to the time it was delivered to the treatment chamber was 10° C. or less for C. botulinum and B. stearothermophilus and 5° C. or less for B. subtilis and B. megatherium; at these temperatures, all the spores remained in the ungerminated, resistant condition up to the instant of radiation.

After irradiation, each outlet bottle connection was clamped and cut from its feed line; one of these bottles was incubated directly without opening. Eight-milliliter portions of other irradiated samples were transferred aseptically to sterile screw-capped tubes, heat-activated, and incubated with unirradiated samples; still other irradiated and control samples were heat-activated and subcultured for count, aerobically in Petri plates or anaerobically in Prickett tubes. In the latter, pork-pea agar [Anderson (2)] and beef-liver infusion agar (NCA) were used. For the aerobes, the counting medium was peptone 5 g., beef extract 3 g., glucose 5 g., soluble starch 1 g., agar 15 g. per liter (pH 7.0). The samples were incubated at optimal temperature for the inoculated organism. Heat activation of the spores was carried out at temperatures ranging from 65 to 98° C., depending upon organism requirements. All of the 62A milk and subculture samples, except the 300-ml. milk bottle, were sealed with sterile sodium thioglycolate agar and mineral oil before incubation.

Spores were considered to have survived treatment if, after irradiation in milk and subculture in nutrient media, they were able to germinate and produce visible growth coloniallv and morphologically similar to that of unirradiated spores and if, after the treatment, irradi-

ated incubated samples underwent spoilage characteristic of the inoculated unirradiated samples similarly incubated. Results are shown in Table 1.

In uninoculated low-spore milk, radiation achieved virtual if not absolute sterility. Since the transfer of irradiated milk to many small tubes necessarily entailed some risk of contamination, an occasional spoiled sample in this (8-ml.) series may be discounted, particularly if unsupported by other evidence of survival. Although the data with *B. subtilis* are inexplicably inconsistent, it appears that an extremely small fraction of the spores survived treatment, at both intensity levels. In the single test with *C. botulinum* at a low but realistic spore level, this organism was not recovered either in the subculture medium or in incubated milk samples, although an aerobic spore-former, presumably present at the time of inoculation, was isolated. Since, in survival determinations with this organism, samples are usually observed for from 6 to 12 mo., the negative result must be accepted with some reservation. Spores of both *B. megatherium* and of *B. stearothermophilus* survived in substantial numbers at each intensity level.

The evident nonlinear relationship between spore survival and radiation intensity may be due to light-screening substances in the milk and/or to heterogeneity of the spore population in respect to radiation resistance. In an effort to shed some light upon the effect of spore clumps on the efficiency of radiation, a suspension of *B. megatherium* spores containing some large and small clumps was divided and one portion shaken vigorously for several minutes with small glass beads. Plate counts were made on both shaken and unshaken samples prior to irradiation. Although shaking of the spore inoculum increased the colony count about 28% and, as indicated by film examinations, materially reduced the size and number of clumps, this reduction in spore clumps did not appreciably increase the lethal effectiveness of the radiation.

The results reported by Albrecht (1), it should be noted, were obtained with raw uninoculated milk, presumably low in spores, comparable in this respect with our pasteurized uninoculated samples. Since, in normally produced milk, neither the kind nor number of spores can be closely controlled, irradiation of uninoculated milk does not furnish a reliable measure of sterilization effectiveness.

CONCLUSIONS

High but substerilization doses of ultraviolet irradiation (Centrifilmer Process) imparted to milk an unpleasant light-activated flavor and odor, which rendered the product unacceptable to critical consumers. Prevention of the flavor change during ultraviolet irradiation of milk, or its later elimination, poses a very difficult problem. Bacterial spores normally present in milk were not all killed by the Centrifilmer Process. Heat sufficient to inactivate lipase in milk intensified the off-flavor which developed during subsequent irradiation. The results were not sufficiently encouraging to continue attempts to produce an acceptable ultraviolet-irradiated, sterilized milk.

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